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(54) Title: TGF-β TYPE RECEPTOR cDNAS AND USES THEREFOR

(57) Abstract

DNA encoding TGF-β type III receptor of mammalian origin, DNA encoding TGF-β type II receptor of mammalian origin, TGF-β type III receptor, TGF-β type II receptor and uses therefor.

-1-

TGF-β TYPE RECEPTOR cDNAs AND USES THEREFOR

Description

Background

Transforming growth factor-beta (TGF-β) is a member
5 of a family of structurally related cytokines that elicit
a variety of responses, including growth, differentia-
tion, and morphogenesis, in many different cell types.
(Roberts, A.B. and M.B. Sporn, In: Peptide Growth
Factors and Their Receptors, Springer-Verlag, Heidelberg,
10 pp. 421-472 (1990); Massague, J., Annu. Rev. Cell. Biol.
6:597-641 (1990)) In vertebrates at least five different
forms of TGF-β, termed TGF-β1 to TGF-β5, have been
identified; they all share a high degree (60%-80%) of
amino-acid sequence identity. While TGF-β1 was initially
15 characterized by its ability to induce anchorage-
independent growth of normal rat kidney cells, its
effects on most cell types are anti-mitogenic. (Altschul,
S.F. et al., J. Mol. Biol. 215:403-410 (1990); Andres,
J.L. et al., J. Cell. Biol. 109:3137-3145 (1989)). It is
20 strongly growth-inhibitory for many types of cells,
including both normal and transformed epithelial, endo-
thelial, fibroblast, neuronal, lymphoid, and hemato-
poietic cells. In addition, TGF-β plays a central role
in regulating the formation of extracellular matrix and
25 cell-matrix adhesion processes.
In spite of its widespread effects on cell phenotype
and physiology, little is known about the biochemical
mechanisms that enable TGF-β family members to elicit
these varied responses. Three distinct high-affinity

-2-

cell-surface TGF-β-binding proteins, termed type I, II
and III, have been identified by incubating cells with
radiolabelled TGF-β1, cross-linking bound TGF-β1 to cell
surface molecules, and analyzing the labelled complexes
by polyacrylamide gel electrophoresis. (Massague, J. and
5   B. Like, J. Biol. Chem. 260:2636-2645 (1985); Cheifetz,
S. et al. J. Biol. Chem. 261:9972-9978 (1986).) The
binding constants are about 5-50pM for the type I and II
receptor and 30-300 pM for the type III receptor. (Boyd,
F.T. and J. Massague, J. Biol. Chem. 264:2272-2278
10   (1989))
The type I and II receptors, of estimated 53 and
70-100 kilodaltons mass respectively, are N-glycosylated
transmembrane proteins that are similar in many respects.
Each of these receptors has a distinct affinity for each
15   member of the TGF-β family of ligands. (Boyd, F.T. and
J. Massague, J. Biol. Chem. 264:2272-2278 (1989)) In
contrast, the type III receptor shows comparable affin-
ities for all TGF-β isotypes; the type III receptor is
the most abundant cell-surface receptor for TGF-β in many
20   cell lines (upwards of 200,000 per cell), and is an
integral membrane proteoglycan. It is heavily modified
by glycosaminoglycan (GAG) groups, and migrates hetero-
geneously upon gel electrophoresis as proteins of 280 to
330 kilodaltons. When deglycosylated with heparitinase
25   and chondrontinase, the protein core migrates as a
100-110 kilodalton protein. The TGF-β binding site
resides in this protein core, as non-glycosylated forms
of this receptor that are produced in cell mutants
defective in GAG synthesis are capable of ligand binding
30   with affinities comparable to those of the natural
receptor. (Cheifetz, S. and J. Massague, J. Biol. Chem.,
264:12025-12028 (1989) A variant form of type III

-3-

receptor is secreted by some types of cells as a soluble
molecule that apparently lacks a membrane anchor. This
soluble species is found in low amounts in serum and in
extracellular matrix.

The type III receptor, also called betaglycan, has a
5 biological function distinct from that of the type I and
II receptors. Some mutant mink lung epithelial cell
(MvlLu) selected for loss of TGF-β responsiveness no
longer express type I receptors; others, similarly
selected, lose expression of both the type I and II
10 receptors. However, all these variants continue to
express the type III receptor. (Boyd, F.T. and J.
Massague, _J. Biol. Chem._ 264:2272-2278 (1989); Laiho, M.
_et al._, _J. Biol. Chem._ 265:18518-18524 (1990)) This has
led to the proposal that types I and II receptors are
15 signal-transducing molecules while the type III receptor,
may subserve some other function, such as in concen-
trating ligand before presentation to the bona fide
signal-transducing receptors. The secreted form of type
III receptor, on the other hand, may act as a reservoir
20 or clearance system for bioactive TGF-β.

Additional information about each of these TGF-β
receptor types would enhance our understanding of their
roles and make it possible, if desired, to alter their
functions.

25 Summary of the Invention
The present invention relates to isolation, sequen-
cing and characterization of DNA encoding the TGF-β type
III receptor of mammalian origin and DNA encoding the
TGF-β type II receptor of mammalian origin. It also
30 relates to the encoded TGF-β type III and type II
receptors, as well as to the soluble form of each; uses

-4-

of the receptor-encoding genes and of the receptors them-
selves; antibodies specific for TGF-β type III receptor
and antibodies specific for TGF-β type II receptor. In
particular, it relates to DNA encoding the TGF-β type III
receptor of rat and human origin, DNA encoding the TGF-β
5   type II receptor of human origin and homologues of each.
    The TGF-β receptor-encoding DNA of the present
invention can be used to identify equivalent TGF-β
receptor type III and type II genes from other sources,
using, for example, known hybridization-based methods or
10 the polymerase chain reaction. The type III receptor
gene, the type II receptor gene or their respective
encoded products can be used to alter the effects of
TGF-β (e.g., by altering receptivity of cells to TGF-β or
interfering with binding of TGF-β to its receptor), such
15 as its effects on cell proliferation or growth, cell
adhesion and cell phenotype. For example, the TGF-β
receptor type III gene, the TGF-β receptor type II gene,
or a truncated gene which encodes less than the entire
receptor (e.g., soluble TGF-β type III receptor, soluble
20 TGF-β type II receptor or the TGF-β type III or type II
binding site) can be administered to an individual in
whom TGF-β effects are to be altered. Alternatively, the
TGF-β type III receptor, the TGF-β type II receptor, a
soluble form thereof (i.e., a form lacking the membrane
25 anchor) or an active binding site of the TGF-β type III
or the type II receptor can be administered to an indivi-
dual to alter the effects of TGF-β.
    Because of the many roles TGF-β has in the body,
availability of the TGF-β receptors described herein
30 makes it possible to further assess TGF-β function
utilizing in vivo as well as in vitro methods and to
alter (enhance or diminish) its effects.

-5-

Brief Description of the Drawings

Figure 1 is the DNA sequence (SEQ ID NO. 1) and the translated amino acid sequence (SEQ ID NO. 2) of type III TGF-β1 receptor cDNA clone R3-OFF (full insert size 6 kb), in which the open reading frame with flanking
5   sequences of the clone are shown. The transmembrane domain is indicated by a single underline. Peptide sequences from purified type III receptor, mentioned in text, that correspond to the derived sequence, are in italics and underlined. Potential N-linked glycosylation
10   sites are indicated by ∮, and extracellular cysteines by &. A consensus protein kinase C phosphorylation site is indicated by $. The last non-vector encoded amino acid of Clone R3-OF (2.9 kb) is indicated by @. Consensus proteoglycan attachment site is indicated by +++. Other
15   potential glycosaminoglycan attachment sites are indicated by +. The upstream in-frame stop codon (-42 to -44) is indicated by a wavy line. Signal peptide cleavage site predicted by vonHeijne's algorithm (von Heijne, G., Nucl. Acid. Res. 14:4683-4690 (1986) is
20   indicated by an arrow.

Figure 2 is the nucleotide sequence of the full-length type II TGF-β receptor cDNA clone 3FF isolated from a human HepG2 cell cDNA library (full insert size 5 kb) (SEQ ID NO. 3). The cDNA has an open reading frame
25   encoding a 572 amino acid residue protein.

Figure 3 is the amino acid sequence of the full-length type II TGF-β receptor (SEQ ID NO. 4).

Detailed Description of the Invention

The subject invention is based on the isolation and
30   sequencing of DNA of vertebrate, particularly mammalian, origin which encodes TGF-β type III receptor and DNA of mammalian origin which encodes TGF-β type II receptor,

-6-

expression of the encoded products and characterization
of the expressed products. As described, a full-length
cDNA which encodes TGF-β receptor type III has been
isolated from a cDNA library constructed from a rat

5   vascular smooth muscle cell line and a full-length cDNA
which encodes TGF-β type II receptor has been isolated
from a human cDNA library. The human homologue of the
type III gene has also been cloned. A deposit of human
TGF-β type III cDNA in the plasmid pBSK has been made

10 under the terms of the Budapest Treaty at the American
Type Culture Collection (10/21/91) under Accession Number
75127. All restrictions upon the availability of the
deposited material will be irrevocably removed upon
granting of a U.S. patent based on the subject

15 application.

        <u>Isolation and Characterization of TGF-β Type III</u>
        <u>Receptor</u>
        As described herein, two separate strategies were
        pursued for the isolation of the TGF-β type III receptor

20 cDNA. In one approach, monoclonal antibodies were
generated against the type III receptor protein and used
to purify the receptor, which was then subjected to
microsequencing. (See Example 1) Microsequencing of
several peptides resulting from partial proteolysis of

25 the purified receptor produced four oligopeptide
sequences, which were used to construct degenerate
oligonucleotides. The degenerate oligonucleotides were
used either as primers in a cloning strategy using the
polymerase chain reaction (PCR) or as probes in screening

30 cDNA libraries. Although this strategy did not prove to
be productive, the oligopeptide sequences were useful in
verifying the identity of the receptor clones isolated by
the second strategy.

-7-

In the second approach to isolating TGF-β receptor-
encoding clones, an expression cloning strategy was used
in COS cells; direct visualization of receptor positive
cells was used to isolate receptor cDNAs. (See Example
2) In this approach, a cDNA library was constructed from
5 A-10 cells, a rat vascular smooth muscle cell line which
expresses all three TGF-β receptors (type I, II and III).
COS cells transfected with cDNA components of this
library in a vector carrying the cytomegalovirus (CMV)
transcriptional promoter and the SV40 origin of repli-
10 cation were screened to identify cells expressing sub-
stantially higher than normal levels of TGF-β receptor.
One transfectant expressing such high levels of a TGF-β
binding protein was identified and the original pool of
expression constructs from which it was derived was split
15 into subpools, which were subjected to a second round of
screening. Two further rounds of sib-selection resulted
in isolation of one cDNA clone (R3-OF) with a 2.9 kb
insert which induced high levels of TGF-β-binding
proteins in approximately 10% of cells into which it was
20 introduced. The specificity of the TGF-β binding was
validated by showing that addition of a 200-fold excess
unlabeled competitor TGF-β1 strongly reduced binding of
125 I-TGF-β to transfected cells.

The R3-OF cDNA encoded an open reading frame of 817
25 amino acid residues, but did not contain a stop codon.
R3-OF was used as a probe to isolate a full-length cDNA
from a rat 208F library. The resulting clone, R3-OFF, is
6kb in length and encodes a protein of 853 amino acids,
which is colinear with clone R3-OF. The nucleotide
30 sequence of R3-OFF is shown in Figure 1, along with the
translated amino acid sequence.

-8-

Characterization of the receptor encoded by R3-OFF
was carried out, as described in Example 3. Results .
showed three distinct TGF-β binding protein species of
TGF-β on the surface of mock-transfected COS cells, which
is in accord with results reported by others. (Massague,
5   J. et al., Ann. NY Acad. Sci. 593:59-72 (1990)). These
included the two lower molecular weight type I and II
receptors (65 and 85 kD) and the higher molecular weight
type III proteoglycan, which migrates as a diffuse band
of 280-330 kd. Enzymatic removal of the proteoglycan
10   yielded a core protein of approximately 100 kd. Binding
to all three receptor types is specific in that it was .
competed by 200-fold excess of unlabeled TGF-β1.
       Transfecting the isolated cDNA caused a two-fold
increase in expression of the type III receptor. When a
15   cell lysate derived from COS cells transfected with clone
R3-OFF was treated with deglycosylating enzymes, the
heterogeneous 280-330 kd band was converted to a protein
core which co-migrates with the type III protein core
seen in parental A10 cells. Importantly, the recombinant
20   protein core migrated differently from the endogenous COS
cell type III protein core.
       These observations were confirmed and extended using
stably transfected cells expressing the type III cDNA.
L6 rat skeleton muscle myoblasts do not express any
25   detectable type III mRNA and no endogeneous surface type
III receptor (Massague et al., 1986; Segarini et al.,
1989). These cells were transfected with the isolated
cDNA in the vector pcDNA-neo. Cell clones stably
expressing this clone in both the forward and reverse
30   orientations with respect to the CMV promoter were
isolated and analyzed by ligand binding assay.

-9-

Introduction of either the full-length clone R3-OFF or the partial clone R3-OF in the forward orientation resulted in expression of type III receptor. L6 cells transfected with the cDNA clones in the reverse orientation did not express this protein. Importantly, the
5   apparent size of the protein core of the type III receptor in cells transformed with the R3-OF clone is smaller than that from R3-OFF transformed cells, consistent with the difference in the sizes of the protein cores predicted from their nucleic acid
10   sequences.

Surprisingly, binding of radio-labeled ligand to the type II receptor was increased by 2.5 fold in cells expressing the type III cDNA. Binding to the type I receptor was unchanged. This apparently specific
15   up-regulation of ligand-binding to the type II receptor was evident in all of the 15 stably transfected L6 cell lines analyzed to date. Furthermore, this effect seems to be mediated equally well by the full-length clone or a truncated clone (R3-OF) that lacks the cytoplasmic domain
20   of TGF-β type III receptor was expressed.

Expression of type III receptor mRNA was assessed by Northern blot analysis and RNA blot analysis. Northern gel analysis showed that the type III receptor mRNA is expressed as a single 6 kb message in several rat
25   tissues. RNA dot blot analysis of several different tissue culture cell lines was also carried out. Cells of mouse origin (MEL and YH16) appear to express a smaller (~5.5 kb) message for the type III mRNA than those of pig, rat and human origin. In all of these cells,
30   expression or absence of the type III mRNA is consistent with the expression or absence of detectable cell surface

-10-

type III receptors, with the notable exception of the
retinoblastoma cell lines (Y79, Weri-1, Weri-24, and
Weri-27). These cells lack detectable surface expression
of type III receptor, which confirms an earlier report.
(Kimchi, A. et al., Science 240:196-198 (1988)). It is
5   striking that the type III receptor mRNA is expressed in
these cells at a level comparable to that of other cells
that do indeed express type III receptor proteins at
readily detectable levels. It appears that TGF-β
receptor III expression, which is substantial in normal
10 retinoblasts (AD12), has been down-regulated in these
retinoblastoma tumor cells, perhaps through post-
transcriptional mechanisms.

     The nucleotide sequence full reading frame along
with flanking sequences of the full-length cDNA clone
15 R3-OFF was determined and is presented in Figure 1. The
reading frame encodes a protein of 853 amino acid
residues, which is compatible with the 100 kD size
observed for the fully deglycosylated TGF-β1 type III
receptor. The identity of the receptor as TGF-β type III
20 was verified by searching for segments of the putative
transcription product which included the peptide
sequences determined by microsequencing of the isolated
type III receptor. (See Example 1) As indicated in
Figure 1, two segments of derived protein (underlined and
25 italicized, residues 378-388 and 427-434) precisely match
with the amino acid sequences of two peptides (I and III)
determined from direct biochemical analysis of the
purified type III receptor.

     Further analysis showed that TGF-β type III binding
30 protein has an unusual structure for a cytokine receptor.
Hydropathy analysis indicates that the protein includes a

-11-

N-terminal signal sequence, followed by a long, hydro-
philic N-terminal region. A 27 residue region of strong
hydrophobicity (underlined in Figure 1, residues 786-812)
toward the C-terminus represents the single putative
transmembrane domain. This suggests that nearly all of

5   the receptor which is an N-terminal extracellular domain
is anchored to the plasma membrane near its C-terminus.
A relatively small C-terminal tail of 41 residues repre-
sents the cytoplasmic domain.

Analysis of related sequences provides few clues to

10   function of TGF-β type III protein. Only one other gene
described to date, a glycoprotein expressed in high
quantities by endothelial cells and termed endoglin,
contains a related amino acid sequence. The most
homologous regions between the sequences of the type III

15   receptor and endoglin (74%) falls primarily in the
putative transmembrane and cytoplasmic domains. Similar
to the general structure of type III receptor, endoglin
is a glycoprotein which contains a large hydrophilic
N-terminal domain which is presumably extracellular,

20   followed by a putative transmembrane domain and a short
cytoplasmic tail of 47 amino acid residues. The bio-
logical role of endoglin is still unclear at present,
although it has been suggested that it may involved in
cell-cell recognition through interactions of an "RGD"

25   sequence on its ectodomain with other adhesion molecules.
Unlike the TGF-β type III receptor, endoglin does not
carry GAG groups.

-12-

Isolation of TGF-β Type II Receptor

The cDNA encoding the type II TGF-β receptor was
also isolated, using expression cloning in COS cells. A
full-length cDNA (designated clone 3FF) was isolated by
high stringency hybridization from a human HepG2 cell
5 cDNA library. (See Example 6) Analysis showed that the
corresponding message is a 5 kb message which is expres-
sed in different cell lines and tissues. Sequence
analysis indicated that the cDNA has an open reading
frame encoding a core 572 amino acid residue protein.
10 The nucleotide sequence of the full-length type II TGF-β
receptor cDNA clone 3FF is shown in Figure 2; the amino
acid sequence is represented in Figure 3.

The 572 amino acid residue protein has a single
putative transmembrane domain, several consensus glyco-
15 sylation sites, and a putative intracellular serine/
threonine kinase domain. The predicted size of the
encoded protein core is -60 kd, which is too large for a
type I TGF-β receptor. Instead, crosslinking experiments
using iodinated TGF-β and COS cells transiently trans-
20 fected with clone 3FF shows over-expression of a protein
approximately 70-80 kd which corresponds to the size of
type II TGF-β receptors. Thus, clone 3FF encodes a
protein that specifically binds TGF-β and has an ex-
pressed protein size of 70-80 kd, both characteristic of
25 the type II TGF-β receptor.

Uses of the Cloned TGF-β Receptors and Related
Products

For the first time, as a result of the work
described herein, DNAs encoding two of the three high
30 affinity cell-surface TGF-β receptors have been isolated,
their sequences and expression patterns determined and

-13-

the encoded proteins characterized. Expression of the
TGF-β type III receptor in cells which do not normally
express the receptor, followed by ligand binding assay,
verified that the cloned type III receptor-encoding DNA
(i.e., either the full-length clone R3-OFF or the partial
5   clone R3-OF) encoded the receptor. In addition, the work
described herein resulted in the surprising finding that
binding of TGF-β to type II receptors in cells expressing
the type III DNA was increased by 2.5 fold.
        Additional insight into the role of the TGF-β type
10   III receptor and its interaction with TGF-β type II
receptor is a result of the work described. For example,
the role of TGF-β type III receptor is unclear, but it
has been proposed that it serves a most unusual function
of attracting and concentrating TGF-βs for eventual
15   transfer to closely situated signal-transducing
receptors. While most cytokines bind to a single cell
surface receptor, members of the TGF-β family bind with
greater or lesser affinity to three distinct cell surface
proteins. This has raised the question of why these
20   three receptors are displayed by most cell types and
whether they subserve distinct functions. Evidence
obtained to date suggests that the type III receptor may
perform functions quite different from those of types I
and II. Thus, type III is substantially modified by GAGs
25   while types I and II appear to carry primarily the
N-linked (and perhaps O-linked) sidechains that are
characteristic of most growth factor receptors. In
addition, variant cells that have been selected for their
ability to resist TGF-β-induced growth inhibition show
30   the absence of Type I or Type II receptors while
continuing to display Type III receptors. Together,
these data have caused some to propose that the Type I

-14-

and II receptors represent <u>bona</u> <u>fide</u> signal-transducing
receptors while the type III receptor, described here,
plays another distinct role in the cell.

It remains possible that the type III receptor
serves a most unusual function of attracting and
5   concentrating TGF-βs on the cell surface for eventual
transfer to closely situated signal-transducing
receptors. Such a function would be unprecedented for a
proteinaceous receptor, although heparin sulfate has been
shown to activate basic FGF by binding to this growth
10   factor prior to FGF association with its receptor (Yayon,
A. <u>et</u> <u>al.</u>, <u>Cell</u> <u>64</u>:841-848 (1991)) Parenthetically,
since the type III receptor also contains large
quantities of heparan sulfate side-chains, it may also
bind and present basic FGF to its receptor.

15   Evidence that is consistent with the role for the
type III receptor comes from the work with L6 rat
myoblast cells which is described herein. As described
above, in L6 cells overexpressing type III receptor, the
binding of radiolabelled TGF-β to the type II receptor is
20   increased several fold when compared with that seen with
parental cells. Further assessment of TGF-β type III
function and interaction with type II and type I
receptors will be needed to answer these questions and
can be carried out using the materials and methods
25   described here.

TGF-β receptors, both type III and type II, can be
identified in other species, using all or a portion of
the DNA encoding the receptor to be identified as a probe
and methods described herein. For example, all or a
30   portion of the DNA sequence encoding TGF-β type III
receptor (shown in Figure 1) or all or a portion of the

-15-

DNA sequence encoding TGF-β type II receptor (shown in
Figure 2) can be used to identify equivalent sequences in
other animals. Stringency conditions used can be varied,
as needed, to identify equivalent sequences in other
species. Once a putative TGF-β receptor type III or type

5   II-encoding sequence has been identified, whether it
encodes the respective receptor type can be determined
using known methods, such as described herein for
verification that the cDNA insert of full-length clone
R3-OFF and the cDNA insert of partial clone R3-OF encode

10   the type III receptor. For example, DNA isolated in this
manner can be expressed in an appropriate host cell which
does not express the receptor mRNA or the surface
receptor (e.g., L6 rat skeleton muscle myoblasts) and
analyzed by ligand binding (TGF-β binding) assay, as

15   described herein.
          Also as a result of the work described herein,
antibodies (polyclonal or monoclonal) specific for the
cloned TGF-β type III or the clones TGF-β type II
receptor can be produced, using known methods. Such

20   antibodies and host cells (e.g., hybridoma cells)
producing the antibodies are also the subject of the
present invention. Antibodies specific for the cloned
TGF-β receptor can be used to identify host cells
expressing isolated DNA thought to encode a TGF-β

25   receptor. In addition, antibodies can be used to block
or inhibit TGF-β activity. For example, antibodies
specific for the cloned TGF-β type III receptor can be
used to block binding of TGF-β to the receptor. They can
be administered to an individual for whom reduction of

30   TGF-β binding is desirable, such as in some fibrotic
diseases (e.g., of skin, kidney and lung).

-16-

The method of the present invention can be used for
diagnosis of disorders involving abnormal binding of .
TGF-β to TGF-β type III receptors and/or TGF-β type II
receptors, such as fibrotic diseases. Abnormal binding
of TGF-β to TGF-β type III receptor or TGF-β type II
5   receptor at a cell surface may be measured, resulting in
a test binding value, which is compared to an appropriate
control binding value. Control binding values can be
obtained using control cells known to have abnormal
binding of TGF-β to its receptors or control cells which
10 are normal cells (e.g., evidence TGF-β binding to the
TGF-β receptor is within physiological levels). Control
values are obtained by determining the extent to which
TGF-β binds the appropriate receptor (i.e., TGF-β type
III receptor or TGF-β type II receptor); such values can
15 be obtained at the time the test binding value is
determined or can be previously determined (i.e., a
previously determined standard). A test binding value
similar to the control binding value obtained from
abnormal cells is indicative of abnormal binding of TGF-β
20 to TGF-β type III receptor or TGF-β type II receptor. A
test binding value similar to the control binding value
obtained from normal cells is indicative of normal
binding of TGF-β to TGF-β type III receptor or TGF-β type
II receptor.
25   DNA and RNA encoding TGF-β type III receptor and DNA
and RNA encoding TGF-β type II receptor are now
available. As used herein, the term DNA or RNA encoding
the respective TGF-β receptor includes any
oligodeoxynucleotide or oligodeoxyribonucleotide sequence
30 which, upon expression, results in production of a TGF-β
receptor having the functional characteristics of the

-17-

TGF-β receptor. That is, the present invention includes
DNA and RNA which, upon expression in an appropriate host
cell, produces a TGF-β type III receptor which has an
affinity for TGF-β similar to that of the TGF-β type III
receptor on naturally occurring cell surfaces (e.g., it
5   shows comparable affinities for all TGF-β isotypes).
Similarly, the present invention includes DNA and RNA
which, upon expression in an appropriate host cell,
produces a TGF-β type II receptor which has an affinity
for TGF-β similar to that of TGF-β type II receptor on
10   naturally occurring cell surfaces (e.g., it has a
distinctive affinity for each member of the TGF-β family
of ligands similar to that of the naturally occurring
TGF-β type II receptor). The DNA or RNA can be produced
in an appropriate host cell or can be produced
15   synthetically (e.g., by an amplification technique such
as PCR) or chemically.
     The present invention also includes the isolated
TGF-β type III receptor encoded by the nucleotide
sequence of full-length R3-OFF, the isolated TGF-β type
20   III receptor encoded by the nucleotide sequence of
partial clone R3-OF, the isolated TGF-β type II receptor
encoded by the nucleotide sequence of full-length clone
3FF and TGF-β type III and type II receptors which bind
TGF-β isotypes with substantially the same affinity. The
25   isolated TGF-β type III and type II receptors can be
produced by recombinant techniques, as described herein,
or can be isolated from sources in which they occur
naturally or synthesized chemically. As used herein, the
terms cloned TGF-β type III and cloned TGF-β type II
30   receptors include the respective receptors identified as

-18-

described herein, and TGF-β type III and type II
receptors (e.g., from other species) which exhibit   ·
substantially the same affinity for the TGF-β isotypes as
the respective receptors.

    As described previously, cells in which the cloned
5   TGF-β type III receptor is expressed bind TGF-β in
essentially the same manner as do cells on which the type
III receptor occurs naturally. Further analysis of
ligand interactions with the cloned TGF-β type III
receptor, based upon site-directed mutagenesis of both
10 TGF-β and the receptor, can be carried out to identify
residues important for binding. For example, DNA having
the sequence of Figure 1 can be altered by adding,
deleting or substituting at least one nucleotide, in
order to produce a modified DNA sequence which encodes a
15 modified cloned TGF-β type III receptor. The functional
characteristics of the modified receptor (e.g., its
TGF-β-binding ability and association of the binding with
effects normally resulting from binding) can be assessed,
using the methods described herein. Modification of the
20 cloned TGF-β type III receptor can be carried out to
produce, for example, a form of the TGF-β type III
receptor, referred to herein as soluble TGF-β receptor,
which is not membrane bound and retains the ability to
bind the TGF-β isotypes with an affinity substantially
25 the same as the naturally-occurring receptor. Such a
TGF-β type III receptor could be produced, using known
genetic engineering or synthetic techniques; it could
include none of the transmembrane region present in the
naturally-occurring TGF-β type III receptor or only a
30 small portion of that region (i.e., small enough not to

-19-

interfere with its soluble nature). For example, it can
include amino acids 1 through 785 of the TGF-β type III
sequence of Figure 1 or a portion of that sequence
sufficient to retain TGF-β binding ability (e.g., amino
acids 24-785, which does not include the signal peptide
5   cleavage site present in the first 23 amino acids). A
soluble TGF-β type II receptor (e.g., one which does not
include the transmembrane and cytoplasmic domains) can
also be produced. For example, it can include amino
acids 1 through 166, inclusive, of Figure 3 or a
10 sufficient portion thereof to retain TGF-β binding
ability substantially the same as that of TGF-β type II
receptor.

    The TGF-β type III receptor and/or type II receptor
can be used for therapeutic purposes. As described above,
15 the TGF-β family of proteins mediate a wide variety of
cellular activities, including regulation of cell growth,
regulation of cell differentiation and control of cell
metabolism. TGF-β may be essential to cell function and
most cells synthesize TGF-β and have TGF-β cell surface
20 receptors. Depending on cell type and environment, the
effects of TGF-β vary: proliferation can be stimulated
or inhibited, differentiation can be induced or
interrupted and cell functions can be stimulated or
suppressed. TGF-β is present from embryonic stages
25 through adult life and, thus, can affect these key
processes throughout life. The similarities of a
particular TGF-β (e.g., TGF-β1) across species and from
cell to cell are considerable. For example, the amino
acid sequence of a particular TGF-β and the nucleotide
30 sequence of the gene which encodes it regardless of

-20-

source, are essentially identical across species. This
further suggests that TGF-β has a critical role in
essential processes.

Specifically, TGF-β has been shown to have anti-
inflammatory and immune suppression capabilities, to play
5   an important role in bone formation (by increasing
osteoblast activity), inhibit cancer cell proliferation
in culture, and control proliferation of glandular cells
of the prostate. As a result, it has potential thera-
peutic applications in altering certain immune system
10 responses (and possibly in modifying immune-mediated
diseases); in treating systemic bone disease (e.g.,
osteoporosis) and conditions in which bone growth is to
be enhanced (e.g., repair of broken bones) and in con-
trolling growth and metastasis of cancer cells. In
15 addition, TGF-β appears to play a role in determining
whether some cell types undergo or do not undergo mito-
sis. In this respect, TGF-β may play an important role
in tissue repair. Some diseases or conditions appear to
involve low production or chronic overproduction of
20 TGF-β. (For example, results of animal studies suggest
that there is a correlation between the over production
of TGF-β and diseases characterized by fibrosis in the
lung, kidney, liver or in viral mediated immune
expression.)
25 Clearly, TGF-β has key roles in body processes and
numerous related potential clinical or therapeutic
applications in wound healing, cancer, immune therapy and
bone therapy. Availability of TGF-β receptor genes, the
encoded products and methods of using them in vitro and
30 in vivo provides an additional ability to control or

-21-

regulate TGF-β activity and effect in the body. For
example, the TGF-β type II or type III receptor encoded
by the type II or the type III receptor genes of the
subject invention can be used, as appropriate, to alter
the effects of TGF-β (e.g., to enhance the effect of
5   TGF-β in the body or to inhibit or reduce (totally or
partially) its effects). It is also possible to admin-
ister to an individual in whom TGF-β bound to TGF-β type
III receptor, such as soluble TGF-β type III receptor.
The present invention provides both a TGF-β agonist and a
10   TGF-β antagonist. For these purposes, DNA gene encoding
the entire TGF-β type II or type III receptor, the
encoded type II or type III receptor or a soluble form of
either receptor can be used. Alternatively, antibodies
or other ligands designed based upon these sequences or
15   specific for them can be used for this purpose.

Knowledge of the amino acid sequences of TGF-β type
III and type II receptors makes it possible to better
understand their structure and to design compounds which
interfere with binding of the receptor with TGF-β. It
20   makes possible identification of existing compounds and
design of new compounds which are type III and/or type II
receptor antagonists.

Cells expressing the type III and/or type II recep-
tors of the present invention can be used to screen
25   compounds for their ability to interfere with (block
totally or partially) TGF binding to the receptors. For
example, cells which do not express TGF-β type III
receptor (e.g., L6 rat skeleton muscle myoblasts) but
have been modified to do so by incorporation of the type
30   III cDNA in an appropriate vector can be used for this

-22-

purpose. A compound to be assessed is added, for
example, to tissue culture dishes containing type III.
expressing cells, along with labeled TGF-β. As a
control, the same concentration of labeled TGF-β is added
5   to tissue culture dishes containing the same type of
cells. After sufficient time for binding of TGF-β to the
receptor to occur, binding of labeled TGF-β to the cells
is assessed, using known methods (e.g., by means of a
gamma counter) and the extent to whcih it occurred in the
10 presence and in the absence of the compound to be
assessed is determined. Comparison of the two values
show whether the test compound blocked TGF-β binding to
the receptor (i.e., less binding in the presence of the
compound than in its absence is evidence that the test
15 compound has blocked binding of TGF-β to the TGF-β type
III receptor).

        Alternatively, a cell line expressing the TGF-β
receptor or cells expressing microinjected TGF-β receptor
RNA, can be used to assess compounds for their ability to
20 block TGF-β binding to the receptor. In this embodiment,
a compound to be assessed is added to tissue culture
dishes containing the cell line cells expressing
microinjected TGF-β receptor RNA, along with TGF-β. As a
control, TGF-β alone is added to the same type of cells
25 expressing microinjected endothelin receptor RNA. After
sufficient time for binding of TGF-β to the receptor to
occur, the extent to which binding occurred is measured,
both in the presence and in the absence of the compound
to be assessed. Comparison of the two values shows
30 whether the compound blocked TGF-β binding to the
receptor. The TGF-β type III and type II receptors can

-23-

also be used to identify TGF-β-like substances, to purify
TGF-β and to identify TGF-β regions which are responsible
for binding to the respective receptors. For example,
the type III receptor can be used in an affinity-based
5   method to identify substances which bind the receptor in
a manner similar to TGF-β.
    The invention will now be illustrated by the
following examples, which are not intended to be limiting
in any way.

10   EXAMPLES

    Materials and methods used in Examples 1-5 are
described below.

Materials
    The following is a description of materials used in
15   the work described herein.
    Recombinant human TGF-β1 was provided by Rik Derynck
of Genentech. COS-M6 cells were provided by Brian Seed
of the Massachusetts General Hospital and Alejandro
Aruffo of Bristol-Myers-Squibb. Heparitinase was pro-
20   vided by Tetsuhito Kojima and Robert Rosenberg of MIT.
LLC-PK$_1$ cells were a gift of Dennis Ausiello of the
Massachusetts General Hospital. YH-16 cell were a gift
of Edward Yeh of the Massachusetts General Hospital. 3-4
cells were a gift of Eugene Kaji of the Whitehead
25   Institute for Biomedical Research. All other cell lines
were purchased from ATCC and grown as specified by the
supplier, except as noted.

-24-

Expression Cloning
Construction of cDNA Library and Generation of
Plasmid Pools

   10μg polyadenylated mRNA was prepared from A10 cells
5 by the proteinase-K/SDS method (Gonda et al., Molec.
   Cell. Biol. 2:617-624 (1982)). Double stranded cDNA was
   synthesized and linkered to nonpalindromic BstX1 adaptors
   as described by Seed, B. and A. Aruffo, Proc. Natl. Acad.
   Sci. USA 84:3365-3369 (1987). Acaptored cDNA was size-
10 fractionated on a 5 to 20% potassium acetate gradient,
   and inserts greater than 1 kb were ligated to the plasmid
   vector pcDNA-1, and electroporated in the E. coli
   MC1061/P3, yielding a primary library with a titer of
   $>10^7$ recombinants. A portion of the cDNA was plated as
15 pools of ~1x10^4 recombinant bacteria colonies grown on 15
   cm petri dishes with Luria-broth agar containing 7.5
   mg/ml tetracycline and 12.5 mg/ml ampicillin. Cells were
   scraped off the plates in 10 mls of Luria-broth, and
   glycerol stocks of pooled bacteria were stored at -70°C.
20 The remaining bacteria was used to purify plasmid DNA
   using the alkaline lysis mini-prep method (Sambrook, J.
   et al., Molecular Cloning: A Laboratory Manual, 2d Ed.
   (Cold Spring Harbor, NY, Cold Spring Harbor Laboratory
   Press (1989)).

25   COS Cell Transfections and Binding Assay
   Plasmid pools (each representing ~1x10^4 clones) were
   transfected into COS-M6 (subclone of COS-7 cells) by the
   DEAE-dextran/chloroquine method described by Seed, B. and
   A. Aruffo, Proc. Natl. Acad. Sci. USA 84:3365-3369
30 (1987). Forty-eight hours after transfection, cells were

-25-

incubated with 50 pM^{125}I-TGF-β1 (100 to 200 Ci/mmol) for
4 hours at 4°C), autoradiographic analysis of transfected
cells was performed using NT-B2 photographic emulsion
(Kodak) essentially as described (Gearing, D.P. et al.,

5   EMBO J. 8:3667-3676 (1989)). After development of
slides, cells were air-dried and mounted with mounting
media and a glass coverslip. Slides were analyzed under
an Olympus OM-T1 inverted phase-contrast microscope using
a dissection trans-illuminator for darkfield illumi-

10 nation.

     Subdivision of Positive Pool
     Of 86 pools screened, one pool (#13) was identified
as positive and a glycerol stock of bacteria corres-
ponding to this pool was titered and 25 pools of 1000

15 clones were generated and miniprep plasmid from these
pools were transfected into COS cells as described above.
Several positive pools of 1000 were identified, and one
was replated as 25 plates of 100 colonies. A replica was
made of this positive plate and harvested. Once a

20 positive pool was identified, individual colonies were
picked from the corresponding master plate and grown
overnight in 3 ml liquid culture. A 2-dimensional grid
representing the 100 clones was generated and a single
clone, R3-OF, was isolated.

25   Cloning of R3-OFF
     A 208F rat fibroblast library in lambda ZAP II
(Stratagene) was screened at high stringency with clone
R3-OF insert, and several clones with ~6kb inserts were
isolated, one of which is referred to as R3-OFF.

-26-

DNA Sequencing and Sequence Analysis

Double-stranded DNA was sequenced by the dideoxy. chain termination method using Sequenase reagents (United States Biochemicals). Comparison of the sequence to the
5   data bases was performed using BLAST (Altschcul, S.F. et al., J. Mol. Biol. 215:403-410 (1990)).

Iodination of TGF-β

TGF-β1 was iodinated using the chloramine-T method as described (Cheifetz, S. and J.L. Andres, J. Biol.
10   Chem. 263:16984-16991 (1988)).

Chemical Cross-Linking

Transfected COS cells grown on 10 cm dishes or subconfluent L6 and A-10 cells grown on 3.5 cm dishes were incubated with ^{125}I-TGF-β1 in binding buffer
15   (Frebs-Ringer buffered with 20 mM Hepes, pH 7.5, 5 mM $MgSO_4$, 0.5% BSA), washed 4 times with ice-cold binding buffer without BSA, and incubated for 15 minutes with binding buffer without BSA containing 60ng/ml disuccinimidyl suberate at 4°C under constant rotation.
20   Crosslinking was terminated by addition of 7% sucrose in binding buffer. Cells were scraped, collected and pelleted by centrifugation, then resuspended in lysis buffer (10 mM Tris, pH 7.4, 1 mM EDTA, pH 8.0, 1% Triton-X 100, 10 μg/ml of pepstatin, 10μg/ml leupeptin,
25   10 μg/ml antipain, 100 μg/m; benzamidine hydrochloride, 100 μg/ml soybean trypsin inhibitor, 50 μg/ml aprotonin, and 1 mM phenylmethylsulfonyl fluoride). Solubilized material was analyzed by 7% SDS-PAGE and subjected to

-27-

autoradiographic analysis by exposure to X-AR film
(Kodak) at -70°C.

Enzymatic Digestion

Digestion of solubilized TGF-b receptors with
5   chondroitinase and heparitinase was performed as
described (Cheifetz, S. and J.L. Andres, _J. Biol. Chem._
263:16984-16991 (1988); Segarini, P.R. and S.M. Seyedin,
J. Biol. Chem., _263_: 8366-8370 (1988).

Generation of Stable Cell Lines

10 .   L6 myoblasts were split 1:10 into 10 cm dishes and
transfected the following day by the calcium phosphate
method (Chen, C. and H. Okayama, _Molec. Cell. Biol._
_7_:2745-2752 (1987)) with clones R3-OF or R3-OFF in the
forward and reverse orientations in the vector pcDNA-neo
15 (InVitrogen).   Cells were subjected to selection in the
presence of G418 (Geneticin, GIBCO) for several weeks
until individual colonies were visible by the naked eye.
These clones were isolated and amplified.

RNA Blot Analyses

20   Rat tissue polyadenylated mRNA was prepared by the
lithium chloride/urea method (Auffrey, C. and F. Raugeon,
Eur. J. Biochemistry _107_:303-313 (1980), followed by
oligo-dT cellulose chromatography (Aviv and Leder, 1972).
Polyadenylated mRNA from cell lines was prepared by the
25 proteinase K/SDS method (Gonda, T.J. _et al._, _Molec. Cell._
Biol. _2_:617-624 (1982)).   Samples of mRNA were resolved
by electrophoresis on 1% agarose-2.2M formaldehyde gels,
blotted onto nylon membranes (Biotrns, ICN) and incubated

-28-

with the 2.9 kb insert of clone Re-OF labeled with ^{32}P by
random priming as probe (Sambrook, J. et al., Molecular
Cloning: A Laboratory Manual, 2d Ed., Cold Spring
Harbor, NY, Cold Spring Harbor Laboratory Press, (1989).
5   Hybridizations were performed at 42°C in hybridization
buffer containing 50% formamide overnight, and blots were
washed at 55°C in 0.2X SSC, 0.1% SDS, before exposure to
X-AR film at -70°C.

     Example 1.   Production of Anti-Type III Receptor Protein
10   Antibodies and Microsequencing and Micro-
                  sequencing of Peptides Resulting from Partial
                  Proteolysis of Purified Type III Receptor
          Initially cellular proteoglycans were purified from
     human placenta and then subjected to enzymatic deglycosy-
15   lation with heparitinase and chondroitinase. Protein
     cores in the molecular weight range of 100-130 kilo-
     daltons were further purified by preparative gel electro-
     phoresis; these should include the type III receptor.
     This partially purified material was used as an immunogen
20   in mice. After screening 850 hybridoma lines developed
     from immunized mice, three lines were found to produce
     antibodies that specifically recognized and immuno-
     precipitated a deglycosylated polypeptide species of
     100-120 kD. This species could be radiolabelled by
25   incubation of whole cells with ^{125}I-TGF-β followed by
     covalent cross-linking. Its size is consistent with that
     of the protein core previously reported for the type III
     receptor. (Massague, J., Annu. Rev. Cell. Biol.
     6:597-641 (1990))

-29-

Monoclonal antibody 94 was used to purify the type
III receptor from rat liver by affinity-chromatography.
The purified receptor was subjected to partial proteo-
lysis and the resulting peptides were resolved by high
5   pressure liquid chromatography. Several peptides were
subjected to microsequencing and yielded the following
oligopeptide sequences:

Peptide I: ILLDPDHPPAL (SEQ ID NO. 5)

Peptide II: QAPFPINFMIA (SEQ ID NO. 6)

10   Peptide III: QPIVPSVQ   (SEQ ID NO. 7)

Peptide IV: FYVEQGYGR   (SEQ ID NO. 8)

These peptide sequences were used to construct
degenerate oligonucleotides that served either as primers
in a cloning strategy using the polymerase chain reaction
15   (PCR) or as probes in screening cDNA libraries. While
this strategy was not productive, the oligopeptide
sequences proved useful in verifying the receptor clones
isolated by the second, alternative strategy (see Example
2).

20   Example 2.   Expression Cloning of the Type III Receptor
                  cDNA
An expression cloning strategy in COS cells, a
procedure which takes advantage of the considerable
amplification of individual cDNAs in transfected COS
25   cells was used as an alternative means to isolate TGF-β
receptor clones. Such amplification is mediated by SV40
large T antigen expressed by the COS cells interacting

-30-

with a SV40 origin of replication in the cDNA vector.
Gearing, D. et al., EMBO J. 8:3667-3676 (1989); Lin,
H.Y., et al., Proc. Natl. Acad. Sci. 88:3185-3189
(1991a); Lin, H. Y. et al., Science, in press (1991);
Mathews, L. S. and Vale, W. W., Cell 65:973-982 (1991).

5   The strategy involved the construction of a cDNA
library from A-10 cells, a rat vascular smooth muscle
cell line that expresses all three high-affinity TGF-β
receptors. The resulting cDNAs were inserted into the
vector pcDNA-1, which carries the CMV transcriptional

10 promoter and the SV40 origin of replication. The result-
ing library was then divided into pools of 10,000 inde-
pendent recombinants each and DNA from each pool was
transfected into 1.5 x 10^6 COS-7 cells grown on glass
flaskettes by means of DEAE-dextran transfection proce-

15 dure. Aruffo, A. and Seed, B., Proc. Natl. Acad. Sci.,
U.S.A. 84:8573-8577 (1987); Gearing, D. et al., EMBO J.
8:3667-3676 (1989); Mathews, L. S. and Vale, W. W., Cell
65:973-982 (1991). The transfected cells were cultured
for 48-60 hours and then exposed to radiolabelled TGF-β1

20 for four hours. Following this treatment, the glass
slides carrying these cells were washed extensively and
fixed. These slides were dipped in liquid photographic
emulsion and examined by darkfield microscopy. While all
of the receptor genes cloned to date by this procedure

25 have undetectable or low constitutive levels of expres-
sion in COS cells, we were hindered by the fact that
untransfected COS cells already express substantial
amounts of type III TGF-β receptor. Such expression,
estimated to be approximately 2 x 10^5 receptor molecules

30 per cell, might well have generated an unacceptably high
level of background binding. However, since the
detection procedure involves visualizing radiolabelled

-31-

ligand-binding on individual cells, it was hoped that
identifying occasional cells expressing substantially
higher levels of vector-encoded receptor would be
possible. This hope was vindicated in the initial
experiments.

5   After screening nearly one million cDNA clones in
this manner, a glass slide containing 20 positive trans-
fectants was identified. The original pool of expression
constructs from which one such transfectant was derived
was split into 25 subpools of 1000 clones each and these

10 were subjected to a second round of screening. Two
further rounds of sib-selection resulted in the isolation
of a cDNA clone (R3-OF) with a 2.9 kb insert that induced
high levels of TGF-β-binding proteins in approximately
10% of COS cells into which it was transfected.

15   The specificity of this binding was validated by
showing that addition of a 200-fold excess of unlabeled
TGF-β competitor strongly reduced binding of ^{125}I-TGF-β
to transfected cells. By taking into account a
transfection efficiency of 10% and the high background of

20 endogenous receptor expression, we calculated that the
level of total ^{125}I-TGF-β binding to each glass slide of
cells transfected with this cDNA clone (Figure 1C) was
only 2-fold above the level seen with mock transfectants
(data not shown). Nonetheless, this marginal increase in

25 ligand-binding was sufficient to identify rare trans-
fectants amidst a large field of cells expressing this
background level of receptor.

The R3-OF cDNA encoded an open reading frame of 836
amino acid residues of which the 3' most 18 were encoded

30 by vector sequence, clearly indicating that clone R3-OF

-32-

was an incomplete cDNA insert which ended prematurely at
the codon specifying alanine 818 (Figure 4). R3-OF was
used as a probe to isolate a full-length cDNA from a rat
208F lambda phage library. This clone, termed R3-OFF,
5   was 6 kb in length and encoded a protein of 853 amino
acids; its sequence was co-linear with that of clone
R3-OF.

Example 3.   Characterization of the Product of the Full
              Length Clone R3-OFF

10   Characterization of the product of the full length
clone R3-OFF was undertaken in order to determine which
of the three TGF-β receptors it specified. To do so, COS
transfectants were incubated with radioiodinated TGF-β,
chemical crosslinker was added and the labelled receptors
15   were resolved by polyacrylamide gel electrophoresis.
          Labelling of cell surface TGF-β receptors in this
way resulted in the detection of three distinct species
on the surface of COS cells, as extensively by others
(Massague, J. et al., Ann. NY Acad. Sci. 593:59-72
20   (1990). These included the two lower molecular weight
type I and II receptors (65 and 85 kD) and the higher
molecular weight type III proteoglycan, which migrated as
a diffuse band of 280-330 kd. Enzymatic treatment of the
proteoglycan with chondroitinase and heparitinase yielded
25   a core protein of approximately 100 kd. Binding to all
three receptor types was specific, in that it was
completed by 200-fold excess of unlabeled TGF-β1.
          Transfecting the R3-OFF cDNA caused a two-fold
increase in expression of the type III receptor.   When a
30   cell lysate derived from COS cells transfected with clone

-33-

R3-OFF was treated with deglycosylating enzymes, the
heterogenous 280-330 kd band was converted to a protein
core which co-migrated with the type III protein core
seen in untransfected A10 cells. Importantly, the
recombinant protein core migrates differently from the
5   endogenous COS cell type III protein core.

These observations were confirmed and extended in
experiments using stably transfected cells expressing the
R3-OFF cDNA. L6 rat skeleton muscle myoblasts normally
do not express detectable type III mRNA or endogenous
10 type III receptor (determined by radiolabelled
ligand-binding assay). Such cells were transfected with
the isolated cDNA in the vector pcDNA-neo. Cell clones
stably expressing this clone in both the forward and
reverse orientations with respect to the CMV promoter
15 were isolated and analyzed by ligand-binding assay.

Introduction of either the full length clone R3-OFF
or the partial clone R3-OF in the forward orientation led
to the de novo expression of the type III receptor. L6
cells transfected with the cDNA in reversed orientation
20 did not express this protein. The apparent size of the
protein core of the type III receptor in cells
transfected with the R3-OF clone is smaller than that
expressed by R3-OFF transfected cells, consistent with
the difference in the sizes of the protein cores
25 predicted from the respective nucleic acid sequences
(Figure 1).

Unexpectedly, the amount of radio-labelled ligand
corss-linked to the type II receptor is increased by 2.5
fold in cells expressing the type III cDNA, while the
30 amount cross-linked to the type I receptor remained

-34-

unchanged. This apparent specific up-regulation of
ligand-binding to the type II receptor could be detected
with all of the 15 stably transfected L6 cell lines
analyzed so far. This effect seems to be mediated by the
5   truncated clone R3-OF which lacks the cytoplasmic domain
as well as by the full-length clone R3-OFF.

Example 4.   Expression of Type III Receptor
         Northern blot analysis demonstrated that the type
III receptor mRNA is expressed as a single 6 kb message
10 in several rat tissues. The level of mRNA expression in
the liver was less than in other tissues, a result
expected from earlier surveys of various tissues using
radioiodinated TGF-β1. Based on this information, it
appears that clone R3-OFF, with a ~6 kb cDNA insert,
15 represents a full length rat type III cDNA clone.
         Cells of mouse origin (MEL and YH16) appear to
express a smaller (~5.5 kb) message for the type III mRNA
than those of pig, rat and human origin. In all of these
cells, expression or absence of the type III mRNA is
20 consistent with the expression or absence of detectable
cell surface type III receptors with the notable
exception of the retinoblastoma cell lines (Y79, Weri-1,
Weri-24, and Weri-27). These cells have previously been
shown to lack detectable surface expression of type III
25 receptor, a result confirmed by our own unpublished work.
It is striking that the type III receptor mRNA is
expressed in these cells at a level comparable to that of
other cells that do indeed express type III receptor
proteins at readily detectable levels. At this moment,
30 we can only conclude that TGF-β receptor III expression,

-35-

which is substantial in normal retinoblasts (AD12), has
been down-regulated in these retinoblastoma tumor cells,
perhaps through post-transcriptional mechanisms.

Example 5.   Sequence Analysis of the Full-Length Type
5   III cDNA
The full-length cDNA clone (R3-OFF), described in
Example 4, was subjected to sequence analysis. The full
reading frame along with flanking sequences is presented
in Figure 1. This reading frame encodes a protein of 853
10 amino acid residues, which is compatible with the 100 kD
observed for the fully deglycosylated TGF-β type III
receptor.
Two segments of derived protein sequence (underlined
and italicized, residues 378-388 and 427-434) precisely
15 match those determined earlier from direct biochemical
analysis of the purified receptor protein. This further
secured the identity of this isolated cDNA clone as
encoding the rat type III receptor.
This TGF-β binding protein has an unusual structure
20 for a cytokine receptor. Hydropathy analysis indicates a
N-terminal signal sequence, followed by a long,
hydrophilic N-terminal region (Kyte, J. and R. F.
Doolittle, J. Mol. Biol. 157:105-132 (1982)). A 27
residue region of strong hydrophobicity (underlined,
25 residues 786-812) toward the C-terminus represents the
single putative transmembrane domain. This suggests that
nearly all of the receptor is composed of an N-terminal
extracellular domain that is anchored to the plasma
membrane near its C-terminus. A relatively short

-36-

C-terminal tail of 41 residues represents the putative
cytoplasmic domain.

        The clone R3-OF was also analyzed and found to be a
truncated version of R3-OFF, with an identical open
reading frame but whose last encoded residue is alanine
5   818 (Figure 1).

        In R3-OFF there are six consensus N-linked
glycosylation sites and 15 cysteines (indicated in Figure
1). There is at least one consensus glycosaminoglycan
addition site at serine 535 (Bernfield, M. and K. C.
10   Hooper, _Ann. N.Y. Acad. Sci._ in press (1991), and
numerous Ser-Gly residues that are potential sites for
GAG conjugation. A consensus protein kinase C site is
also present at residue 817.

        Only one other gene described to date, a
15   glycoprotein expressed in high quantities by endothelial
cells and termed endoglin (Gougos and Letarte, 1990),
contains a related amino acid sequence. Overall, there
is ~30% identity with the type III receptor over the
entire 645 amino acid residue length of endoglin. The
20   most homologous regions between the sequences of the type
III receptor and endoglin (74% identity) falls primarily
in the putative transmembrane and cytoplasmic domains.
Similar to the general structure of type III receptor,
endoglin is a glycoprotein which contains a large
25   hydrophilic and presumably extracellular N-terminal
domain followed by a putative transmembrane domain and a
short cytoplasmic tail of 47 amino acid residues. The
biological role of endoglin is unclear, though it has
been suggested that it may involve cell-cell recognition
30   through interactions of an "RGD" sequence on its

-37-

ectodomain with other adhesion molecules. Unlike the
TGF-β type III receptor, endoglin does not carry GAG
groups.

Equivalents

5   Those skilled in the art will recognize, or be able
to ascertain using not more than routine experimentation,
many equivalents to the specific embodiments of the
invention described herein. Such equivalents are
intended to be encompassed by the following claims.

International Application No: PCT/

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page ____6____, line ___12___ of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet ☐ [3]

Name of depositary institution [4]

American Type Culture Collection

Address of depositary institution (including postal code and country) [4]

12301 Parklawn Drive
Rockville, Maryland 20852 USA

Date of deposit [5]	Accession Number [6]
21 October 1991	75127

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European Patent is sought, the Applicant hereby informs the European Patent Office under European Rule 28(4) that, until the publication of the mention of the grant of the European Patent or until the date on which the European Application has been refused or is withdrawn or is deemed to be withdrawn, the availability of the biological material deposited with the American Type Culture Collection under Accession No.
shall be effected only by the issue of a sample to an expert nominated by the requester in accordance with European Rule 28(5).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

Europe (EP)
Australia
Canada
Japan

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g. "Accession Number of Deposit")

. . . .

E. ☑ This sheet was received with the international application when filed (to be checked by the receiving Office)

FRANZ L. BOENER
INTERNATIONAL DIVISION
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

___
(Authorized Officer)

Form PCT/RO/134 (January 1981)

-39-

<u>CLAIMS</u>

1.   Isolated DNA encoding TGF-β receptor of vertebrate
      origin or DNA which hybridizes thereto and encodes
      TGF-β receptor of vertebrate origin.

5   2.   Isolated DNA of Claim 1 wherein the TGF-β receptor
           is TGF-β type III receptor or TGF-β type II
           receptor.

3.   Isolated DNA of Claim 2 which is of mammalian
      origin.

10   4.   Isolated DNA of murine or human origin encoding
           TGF-β type III receptor or DNA which hybridizes
           thereto.

5.   Isolated DNA of Claim 4 having the nucleotide
      sequence of Figure 1 or a portion thereof sufficient
15   to encode TGF-β type III receptor.

6.   Isolated DNA of murine or human origin encoding
      TGF-β type II receptor or DNA which hybridizes
      thereto.

7.   Isolated DNA of Claim 6 having the nucleotide
20   sequence of Figure 2 or a portion thereof sufficient
      to encode TGF-β type II receptor.

8.   Isolated TGF-β type III receptor of mammalian
      origin.

- 40 -

9.   Isolated TGF-β type III receptor of Claim 8 having
      the amino acid sequence of Figure 1 or a
      substantially similar amino acid sequence.

10.   Isolated TGF-β type II receptor of mammalian origin.

5   11.   Isolated TGF-β type II receptor of Claim 10 having
          the amino acid sequence of Figure 3 or a
          substantially similar amino acid sequence.

12.   Recombinant TGF-β type III receptor of mammalian
      origin.

10 13.   Recombinant TGF-β type III receptor of Claim 8
          having the amino acid sequence of Figure 1 or a
          substantially similar amino acid sequence.

14.   Recombinant TGF-β type II receptor of mammalian
      origin.

15 15.   Recombinant TGF-β type II receptor of Claim 10
          having the amino acid sequence of Figure 4 or a
          substantially similar amino acid sequence.

16.   Soluble TGF-β receptor.

17.   Soluble TGF-β receptor of Claim 16 which is soluble
20   TGF-β type III receptor.

18.   Soluble TGF-β type III receptor of Claim 17 in which
      the amino acid sequence is amino acids 1 through

-41-

785, inclusive, of Figure 1 or a substantially
similar amino acid sequence.

19. Soluble TGF-β receptor of Claim 16 which is soluble
     TGF-β type II receptor.

5   20. Soluble TGF-β receptor of Claim 19 in which the
         amino acid sequence is approximately amino acids 1
         through 166, inclusive, of Figure 3, or a
         substantially similar amino acid sequence.

    21. An antibody which specifically recognized TGF-β type
10   III receptor of mammalian origin.

    22. An antibody of Claim 21 which is a monoclonal
         antibody.

    23. An antibody which specifically recognizes soluble
         TGF-β type III receptor of mammalian origin.

15 24. An antibody which specifically recognizes soluble
         TGF-β type II receptor of mammalian origin.

    25. A method of altering TGF-β binding to TGF-β type II
         or type III receptor on the surface of a cell,
         comprising combining soluble TGF-β type II or type
20   III receptor with the cell, under conditions
         appropriate for binding of the soluble TGF-β
         receptor and TGF-β.

-42-

26. The method of Claim 25 wherein TGF-β binding is inhibited.

27. A method of altering TGF-β binding to TGF-β type III receptor on the surface of a cell comprising combining the cell with DNA encoding TGF-β type III receptor in an appropriate expression system which expresses TGF-β type III receptor, under conditions appropriate for expression of TGF-β type III receptor and binding of TGF-β with TGF-β type III receptor.

28. A method of regulating the effect of TGF-β in a mammal, comprising administering to the mammal a TGF-β receptor selected from the group consisting of: TGF-β type III receptor, TGF-β type II receptor, soluble TGF-β type III receptor, soluble TGF-β type II receptor, TGF-β bound to TGF-β type III receptor or a combination thereof, in sufficient quantity to alter binding of TGF-β to TGF-β type III receptor, binding of TGF-β to TGF-β type II receptor or both.

-43-

29.   TGF-β receptor according to any one of Claims 8 to 20, for use in therapy.

30.   An antibody according to any one of Claims 21 to 24, for use in therapy.

5   31.   Use of TGF-β receptor according to any one of Claims 8 to 20, for the manufacture of a medicament for altering (e.g. inhibiting) TGF-β binding to TGF-β type II or type III receptor on the surface of a cell.

10   32.   Use of a TGF-β receptor selected from the group consisting of: TGF-β type III receptor, TGF-β type II receptor, soluble TGF-β type III receptor, soluble TGF-β type II receptor, TGF-β bound to TGF-β type III receptor or a combination thereof, for the 15   manufacturing of a medicament for use in regulating the affect of TGF-β in a mammal.

33.   A method of assessing the ability of a compound to interfere with TGF-β binding to the TGF-β type III receptor, comprising the steps of:

20   a)   combining:

        1)   mammalian cells which express the TGF-β type III receptor;

        2)   labeled TGF-β; and

        3)   a compound to be assessed;

-44-

b)   maintaining the product of (a) under conditions
        sufficient for TGF-β to bind to the TGF-β type
        III receptor;

c)   determining the extent of binding of TGF-β to
        TGF-β type III receptors in the presence of the
        compound to be assessed; and

d)   comparing the determination made in (c) with
        the extent to which binding of TGF-β to the
        TGF-β type III receptor occurs in the absence
        of the compound to be assessed,
wherein if TGF-β binding to the TGF-β type III
receptor occurs to a lesser extent in the presence
of the compound to be assessed than in the absence
of the compound to be assessed, the compound to be
assessed interferes with TGF-β binding to TGF-β type
III receptors.

34.   A method of Claim 33 wherein the cells which express
        the TGF-β type III receptor are a cell line.

35.   A method of Claim 34 wherein the cells which express
        the TGF-β type III receptor are cells modified to
        express the TGF-β type III receptor.

36.   A method of Claim 35 wherein the cells modified to
        express the TGF-β type III receptor are cells which
        have incorporated into them TGF-β receptor cDNA in
        an appropriate vector or microinjected TGF-β
        receptor RNA.

-45-

37. A method of assessing the ability of a compound to
     interfere with TGF-β binding to the TGF-β type II
     receptor comprising the steps of:
     a)   combining:
          1)   mammalian cells which express the TGF-β
               type II receptor;
          2)   labeled TGF-β; and
          3)   a compound to be assessed;
     b)   maintaining the product of (a) under conditions
          sufficient for TGF-β to bind to the TGF-β type
          II receptor;
     c)   determining the extent of binding of TGF-β to
          TGF-β type II receptors in the presence of the
          compound to be assessed; and
     d)   comparing the determination made in (c) with
          the extent to which binding of TGF-β to the
          TGF-β type II receptor occurs in the absence of
          the compound to be assessed,
     wherein if TGF-β binding to the TGF-β type II
     receptor occurs to a lesser extent in the presence
     of the compound to be assessed than in the absence
     of the compound to be assessed, the compound to be
     assessed has interfered with TGF-β binding to TGF-β
     type II receptor.

38. A method of Claim 37 wherein the cells which express
     the TGF-β type II receptor are a cell line.

39. A method of Claim 38 wherein the cells which express
     the TGF-β type II receptor are cells modified to
     express the TGF-β type II receptor.

-46-

40. A method of Claim 39 wherein the cells modified to express the TGF-β type II receptor are cells which have incorporated into them TGF-β receptor cDNA in an appropriate vector or microinjected TGF-β

5   receptor RNA.

41. A method of detecting abnormal binding of TGF-β TGF-β type III receptors of TGF-β type II receptors at a cell surface, comprising:

   a) determining the extent of binding of TGF-β to
10   TGF-β type III receptors or TGF-β type II receptors by cells in a sample obtained from an individual in whom binding is to be assessed thereby producing a test binding value; and

   b) comparing the results of (a) with the extent to
15   which binding occurs at the cell surface in control cells which are cells known to have abnormal binding of TGF-β to TGF-β type III receptors or TGF-β type II receptors resulting in a control binding value,

20   wherein a test binding value similar to the control binding value is indicative of abnormal binding of TGF-β to TGF-β type III receptor or TGF-β type II receptor.

1/7

FIGURE 1A

-240
CAGGAGGTGA AAGTCCCCGG CGGTCCGGAT GGCGCAGTTG CACTGCGCTG CTGAGCTCGC -180
GGCCGCCTGC GCACACTGGG GGGACTCGCT TCGGCTAGTA ACTCCTCCAC CTCGCGGCGG -120
ACGACCGGTC CTGGACACGC TGCCTGCGAG GCAAGTTGAA CAGTGCAGAG AAGGATCTTA - 60
AAGCTACACC CGACTTGCCA CGATTGCCTT CAATCTGAAG AACCAAAGGC TGTTGGAGAG - 1

ATG GCA GTG ACA TCC CAC CAC ATG ATC CCG GTG ATG GTT GTC CTG ATG   48
Met Ala Val Thr Ser His His Met Ile Pro Val Met Val Val Leu Met   16

AGC GCC TGC CTG GCC ACC GCC GGT CCA GAG CCC AGC ACC CGG TGT GAA   96
Ser Ala Cys Leu Ala Thr Ala Gly Pro Glu Pro Ser Thr Arg Cys Glu   32
                            ↑   &

CTG TCA CCA ATC AAC GCC TCT CAC CCA GTC CAG GCC TTG ATG GAG AGC   144
Leu Ser Pro Ile Asn Ala Ser His Pro Val Gln Ala Leu Met Glu Ser   48
                    #

TTC ACC GTT CTG TCT GGC TGT GCC AGC AGA GGC ACC ACC GGG CTG CCA   192
Phe Thr Val Leu Ser Gly Cys Ala Ser Arg Gly Thr Thr Gly Leu Pro   64
                        +   &

AGG GAG GTC CAT GTC CTA AAC CTC CGA AGT ACA GAT CAG GGA CCA GGC   240
Arg Glu Val His Val Leu Asn Leu Arg Ser Thr Asp Gln Gly Pro Gly   80

CAG CGG CAG AGA GAG GTT ACC CTG CAC CTG AAC CCC ATT GCC TCG GTG   288
Gln Arg Gln Arg Glu Val Thr Leu His Leu Asn Pro Ile Ala Ser Val   96

CAC ACT CAC CAC AAA CCT ATC GTG TTC CTG CTC AAC TCC CCC AGG CCC   336
His Thr His His Lys Pro Ile Val Phe Leu Leu Asn Ser Pro Gln Pro   112

CTG GTG TGG CAT CTG AAG ACG GAG AGA CTG GCC GCT GGT GTC CCC AGA   384
Leu Val Trp His Leu Lys Thr Glu Arg Leu Ala Ala Gly Val Pro Arg   128

CTC TTC CTG GTT TCG GAG GGT TCT GTG GTC CAG TTT CCA TCA GGA AAC   432
Leu Phe Leu Val Ser Glu Gly Ser Val Val Gln Phe Pro Ser Gly Asn   144
                                                +   #

TTC TCC TTG ACA GCA GAA ACA GAG GAA AGG AAT TTC CCT CAA GAA AAT   480
Phe Ser Leu Thr Ala Glu Thr Glu Glu Arg Asn Phe Pro Gln Glu Asn   160

GAA CAT CTC GTG CGC TGG GCC CAA AAG GAA TAT GGA GCA GTG ACT TCG   528
Glu His Leu Val Arg Trp Ala Gln Lys Glu Tyr Gly Ala Val Thr Ser   176

TTC ACT GAA CTC AAG ATA GCA AGA AAC ATC TAT ATT AAA GTG GGA GAA   576
Phe Thr Glu Leu Lys Ile Ala Arg Asn Ile Tyr Ile Lys Val Gly Glu   192

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2/7

FIGURE 1B

```
GAT CAA GTG TTT CCT CCT ACG TGT AAC ATA GGG AAG AAT TTC CTC TCA      624
Asp Gln Val Phe Pro Pro Thr Cys Asn Ile Gly Lys Asn Phe Leu Ser      208
                                &


CTC AAT TAC CTT GCC GAG TAC CTT CAA CCC AAA GCC GCC GAA GGT TGT      672
Leu Asn Tyr Leu Ala Glu Tyr Leu Gln Pro Lys Ala Ala Glu Gly Cys      224
                                                                &


GTC CTG CCC AGT CAG CCC CAT GAA AAG GAA GTA CAC ATC ATC GAG TTA      720
Val Leu Pro Ser Gln Pro His Glu Lys Glu Val His Ile Ile Glu Leu      240


ATT ACC CCC AGC TCG AAC CCT TAC AGC GCT TTC CAG GTG GAT ATA ATA      768
Ile Thr Pro Ser Ser Asn Pro Tyr Ser Ala Phe Gln Val Asp Ile Ile     256


GTT GAC ATA CGA CCT GCT CAA GAG GAT CCC GAG GTG GTC AAA AAC CTT      816
Val Asp Ile Arg Pro Ala Gln Glu Asp Pro Glu Val Val Lys Asn Leu      272


GTC CTG ATC TTG AAG TGC AAA AAG TCT GTC AAC TGG GTG ATC AAG TCT      864
Val Leu Ile Leu Lys Cys Lys Lys Ser Val Asn Trp Val Ile Lys Ser      288
                    &


TTT GAC GTC AAG GGA AAC TTG AAA GTC ATT GCT CCC AAC AGT ATC GGC      912
Phe Asp Val Lys Gly Asn Leu Lys Val Ile Ala Pro Asn Ser Ile Gly      304


TTT GGA AAA GAG AGT GAA CGA TCC ATG ACA ATG ACC AAA TTG GTA AGA      960
Phe Gly Lys Glu Ser Glu Arg Ser Met Thr Met Thr Lys Leu Val Arg      320


GAT GAC ATC CCT TCC ACC CAA GAG AAT CTG ATG AAG TGG GCA CTG GAC     1008
Asp Asp Ile Pro Ser Thr Gln Glu Asn Leu Met Lys Trp Ala Leu Asp      336


AAT GGC TAC AGG CCA GTG ACG TCA TAC ACA ATG GCT CCC GTG GCT AAT     1056
Asn Gly Tyr Arg Pro Val Thr Ser Tyr Thr Met Ala Pro Val Ala Asn      352


AGA TTT CAT CTT CGG CTT GAG AAC AAC GAG GAG ATG AGA GAT GAG GAA     1104
Arg Phe His Leu Arg Leu Glu Asn Asn Glu Glu Met Arg Asp Glu Glu      368


GTC CAC ACC ATT CCT CCT GAG CTT CGT ATC CTG CTG GAC CCT GAC CAC     1152
Val His Thr Ile Pro Pro Glu Leu Arg Ile Leu Leu Asp Pro Asp His      384
                                          peptide 1


CCG CCC GCC CTG GAC AAC CCA CTC TTC CCA GGA GAG GGA AGC CCA AAT     1200
Pro Pro Ala Leu Asp Asn Pro Leu Phe Pro Gly Glu Gly Ser Pro Asn      400


GGT GGT CTC CCC TTT CCA TTC CCG GAT ATC CCC AGG AGA GGC TGG AAG     1248
Gly Gly Leu Pro Phe Pro Phe Pro Asp Ile Pro Arg Arg Gly Trp Lys      416


GAG GGC GAA GAT AGG ATC CCC CGG CCA AAG CAG CCC ATC GTT CCC AGT     1296
Glu Gly Glu Asp Arg Ile Pro Arg Pro Lys Gln Pro Ile Val Pro Ser      432
                                          peptide 2
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3/7

FIGURE 1C

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GTT CAA CTG CTT CCT GAC CAC CGA GAA CCA GAA GAA GTG CAA GGG GGC    1344
Val Gln Leu Leu Pro Asp His Arg Glu Pro Glu Glu Val Gln Gly Gly     448

GTG GAC ATC GCC CTG TCA GTC AAA TGT GAC CAT GAA AAG ATG GTC GTG    1392
Val Asp Ile Ala Leu Ser Val Lys Cys Asp His Glu Lys Met Val Val     464
                                    &

GCT GTA GAC AAA GAC TCT TTC CAG ACC AAT GGC TAC TCA GGG ATG GAG    1440
Ala Val Asp Lys Asp Ser Phe Gln Thr Asn Gly Tyr Ser Gly Met Glu     480
                                                    +

CTC ACC CTG TTG GAT CCT TCG TGT AAA GCC AAA ATG AAT GGT ACT CAC    1488
Leu Thr Leu Leu Asp Pro Ser Cys Lys Ala Lys Met Asn Gly Thr His     496
                              &                      #

TTT GTT CTC GAG TCT CCC CTG AAT GGC TGT GGT ACT CGA CAT CGG AGG    1536
Phe Val Leu Glu Ser Pro Leu Asn Gly Cys Gly Thr Arg His Arg Arg     512
                                        &

TCG ACC CCG GAT GGT GTG GTT TAC TAT AAC TCT ATT GTG GTG CAG GCT    1584
Ser Thr Pro Asp Gly Val Val Tyr Tyr Asn Ser Ile Val Val Gln Ala     528

CCG TCC CCT GGG GAT AGC AGT GGC TGG CCT GAT GGC TAT GAA GAC TTG    1632
Pro Ser Pro Gly Asp Ser Ser Gly Trp Pro Asp Gly Tyr Glu Asp Leu     544
                    +++

GAG TCA GGC GAT AAT GGA TTT CCT GGA GAC GGG GAT GAA GGA GAA ACT    1680
Glu Ser Gly Asp Asn Gly Phe Pro Gly Asp Gly Asp Glu Gly Glu Thr     560
        +

GCC CCC CTG AGC CGA GCT GGA GTG GTG GTG TTT AAC TGC AGC TTG CGG    1728
Ala Pro Leu Ser Arg Ala Gly Val Val Val Phe Asn Cys Ser Leu Arg     576
                                            #   &

CAG CTG AGG AAT CCC AGT GGC TTC CAG GGC CAG CTC GAT GGA AAT GCT    1776
Gln Leu Arg Asn Pro Ser Gly Phe Gln Gly Gln Leu Asp Gly Asn Ala     592
                                                        #

ACC TTC AAC ATG GAG CTG TAT AAC ACA GAC CTC TTT CTG GTG CCC TCC    1824
Thr Phe Asn Met Glu Leu Tyr Asn Thr Asp Leu Phe Leu Val Pro Ser     608

CCA GGG GTC TTC TCT GTG GCA GAG AAC GAG CAT GTT TAT GTT GAG GTG    1872
Pro Gly Val Phe Ser Val Ala Glu Asn Glu His Val Tyr Val Glu Val     624

TCT GTC ACC AAG GCT GAC CAA GAT CTG GGA TTC GCC ATC CAA ACC TGC    1920
Ser Val Thr Lys Ala Asp Gln Asp Leu Gly Phe Ala Ile Gln Thr Cys     640
                                                            &
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4/7

FIGURE 1D

```
TTT CTC TCT CCA TAC TCC AAC CCA GAC AGA ATG TCT GAT TAC ACC ATC    1968
Phe Leu Ser Pro Tyr Ser Asn Pro Asp Arg Met Ser Asp Tyr Thr Ile     656

ATC GAG AAC ATC TGT CCG AAA GAC GAC TCT GTG AAG TTC TAC AGC TCC    2016
Ile Glu Asn Ile Cys Pro Lys Asp Asp Ser Val Lys Phe Tyr Ser Ser     672
                 &

AAG AGA GTG CAC TTT CCC ATC CCG CAT GCT GAG GTG GAC AAG AAG CGC    2064
Lys Arg Val His Phe Pro Ile Pro His Ala Glu Val Asp Lys Lys Arg     688

TTC AGC TTC CTG TTC AAG TCT GTG TTC AAC ACC TCC CTG CTC TTC CTG    2112
Phe Ser Phe Leu Phe Lys Ser Val Phe Asn Thr Ser Leu Leu Phe Leu     704
                                             #

CAC TGC GAG TTG ACT CTG TGC TCC AGG AAG AAG GGC TCC CTG AAG CTG    2160
His Cys Glu Leu Thr Leu Cys Ser Arg Lys Lys Gly Ser Leu Lys Leu     720
       &                       &

CCG AGG TGT GTG ACT CCT GAC GAC GCC TGC ACT TCT CTC GAT GCC ACC    2208
Pro Arg Cys Val Thr Pro Asp Asp Ala Cys Thr Ser Leu Asp Ala Thr     736
        &                               &

ATG ATC TGG ACC ATG ATG CAG AAT AAG AAG ACA TTC ACC AAG CCC CTG    2256
Met Ile Trp Thr Met Met Gln Asn Lys Lys Thr Phe Thr Lys Pro Leu     752

GCT GTG GTC CTC CAG GTA GAC TAT AAA GAA AAT GTT CCC AGC ACT AAG    2304
Ala Val Val Leu Gln Val Asp Tyr Lys Glu Asn Val Pro Ser Thr Lys     768

GAT TCC AGT CCA ATT CCT CCT CCT CCT CCA CAG ATT TTC CAT GGC CTG    2352
Asp Ser Ser Pro Ile Pro Pro Pro Pro Pro Gln Ile Phe His Gly Leu     784

GAC ACG CTC ACC GTG ATG GGC ATT GCA TTT GCA GCA TTT GTG ATC GGA    2400
Asp Thr Leu Thr Val Met Gly Ile Ala Phe Ala Ala Phe Val Ile Gly     800

GCG CTC CTG ACG GGG GCC TTG TGG TAC ATC TAC TCC CAC ACA GGG GAG    2448
Ala Leu Leu Thr Gly Ala Leu Trp Tyr Ile Tyr Ser His Thr Gly Glu     816

ACA GCA CGA AGG CAG CAA GTC CCT ACC TCG CCG CCA GCC TCG GAG AAC    2496
Thr Ala Arg Arg Gln Gln Val Pro Thr Ser Pro Pro Ala Ser Glu Asn     832
     $   @

AGC AGC GCG GCC CAC AGC ATC GGC AGC ACT CAG AGT ACC CCC TGC TCT    2544
Ser Ser Ala Ala His Ser Ile Gly Ser Thr Gln Ser Thr Pro Cys Ser     848

AGC AGC AGC ACA GCC TAGGTGGACA GACAGACGCC CGCCCACCGC AGCCAGGGCA    2599
Ser Ser Ser Thr Ala***                                              853
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FIGURE 1E

GGGCCCGATG CCAGTGCTGC GTGTCCACAG TCAGAAGTCT TGATCTGGGC TCCCTGTAAA 2659
GAAAGAGTGA ATTTCAGTAT ACAGACAGCC AGTTCTACCC ACCCCTTACC ACGGCCCACA 2719
TAAATGTGAC CCTGGGCATC TGTCACACGA AAGCTAAGCT GGTGGCCTTC CCCACCAGCC 2779
CCTCGCAGGA TGGGGGTTTC AATGTGAAAC ATCTGCCAGT TTTGTTTTGT TTTTTTAATG 2839
CTGCTTTGTC CAGGTGTCCA AACATCCATC ATTTGGGGTG GTCTGTTTTA CAGAGTAAAG 2899
GAGGCGGTGA AGGGACGTCA GCTAGTGTGT AGAGCCAAGG GGAGACAGCT AGGATTCTCG 2959
CCTAGCTGAA CCAAGGTGTA AAATAGAAGA CACGCTCC   2997

6/7

FIGURE 2

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                                                              GTTGGCGAGG
AGTTTCCTGT  TTCCCCCGCA  GCGCTGAGTT  GAAGTTGAGT  GAGTCACTCG
CGCGCACGGA  GCGACGACAC  CCCCGCGCGT  GCACCCGCTC  GGGACAGGAG
CCGGACTCCT  GTGCAGCTTC  CCTCGGCCGC  CGGGGGCCTC  CCCGCGCCTC
GCCGGCCTCC  AGGCCCTCC   TGGCTGGCGA  GCGGGCGCCA  CATCTGGCCC
GCACATCTGC  GCTGCCGGCC  CGGCGCGGGG  TCCGGAGAGG  GCGCGGCGCG
GAGCGCAGCC  AGGGGTCCGG  GAAGGCGCCG  TCCGTGCGCT  GGGGGCTCGG
TCTATGACGA  GCAGCGGGGT  CTGCCATGGG  TCGGGGGCTG  CTCAGGGGCC
TGTGGCCGCT  GCACATCGTC  CTGTGGACGC  GTATCGCCAG  CACGATCCCA
CCGCACGTTC  AGAAGTCGGT  TAATAACGAC  ATGATAGTCA  CTGACAACAA
CGGTGCAGTC  AAGTTTCCAC  AACTGTGTAA  ATTTGTGAT   GTGAGATTTT
CCACCTGTGA  CAACCAGAAA  TCCTGCATGA  GCAACTGCAG  CATCACCTCC
ATCTGTGAGA  AGCCACAGGA  AGTCTGTGTG  GCTGTATGGA  GAAAGAATGA
CGAGAACATA  ACACTAGAGA  CAGTTTGCCA  TGACCCCAAG  CTCCCCTACC
ATGACTTTAT  TCTGGAAGAT  GCTGCTTCTC  CAAAGTGCAT  TATGAAGGAA
AAAAAAAAGC  CTGGTGAGAC  TTTCTTCATG  TGTTCCTGTA  GCTCTGATGA
GTGCAATGAC  AACATCATCT  TCTCAGAAGA  ATATAACACC  AGCAATCCTG
ACTTGTTGCT  AGTCATATTT  CAAGTGACAG  GCATCAGCCT  CCTGCCACCA
CTGGGAGTTG  CCATATCTGT  CATCATCATC  TTCTACTGCT  ACCGCGTTAA
CCGGCAGCAG  AAGCTGAGTT  CAACCTGGGA  AACCGGCAAG  ACGCGGAAGC
TCATGGAGTT  CAGCGAGCAC  TGTGCCATCA  TCCTGGAAGA  TGACCGCTCT
GACATCAGCT  CCACGTGTGC  CAACAACATC  AACCACAACA  CAGAGCTGCT
GCCCATTGAG  CTGGACACCC  TGGTGGGGAA  AGGTCGCTTT  GCTGAGGTCT
ATAAGGCCAA  GCTGAAGCAG  AACACTTCAG  AGCAGTTTGA  GACAGTGGCA
GTCAAGATCT  TTCCCTATGA  GGAGTATGCC  TCTTGGAAGA  CAGAGAAGGA
CATCTTCTCA  GACATCAATC  TGAAGCATGA  GAACATACTC  CAGTTCCTGA
CGGCTGAGGA  GCGGAAGACG  GAGTTGGGGA  AACAATACTG  GCTGATCACC
GCCTTCCACG  CCAAGGGCAA  CCTACAGGAG  TACCTGACGC  GGCATGTCAT
CAGCTGGGAG  GACCTGCGCA  AGCTGGGCAG  CTCCCTCGCC  CGGGGGATTG
CTCACCTCCA  CAGTGATCAC  ACTCCATGTG  GGAGGCCCAA  GATGCCCATC
GTGCACAGGG  ACCTCAAGAG  CTCCAATATC  CTCGTGAAGA  ACGACCTAAC
CTGCTGCCTG  TGTGACTTTG  GGCTTTCCCT  GCGTCTGGAC  CCTACTCTGT
CTGTGGATGA  CCTGGCTAAC  AGTGGGCAGG  TGGGAACTGC  AAGATACATG
GCTCCAGAAG  TCCTAGAATC  CAGGATGAAT  TTGGAGAATG  CTGAGTCCTT
CAAGCAGACC  GATGTCTACT  CCATGGCTCT  GGTGCTCTGG  GAAATGACAT
CTCGCTGTAA  TGCAGTGGGA  GAAGTAAAAG  ATTATGAGCC  TCCATTTGGT
TCCAAGGTGC  GGGAGCACCC  CTGTGTCGAA  AGCATGAAGG  ACAACGTGTT
GAGAGATCGA  GGGCGACCAG  AAATTCCCAG  CTTCTGGCTC  AACCACCAGG
GCATCCAGAT  GGTGTGTGAG  ACGTTGACTG  AGTGCTGGGA  CCACGACCCA
GAGGCCCGTC  TCACAGCCCA  GTGTGTGGCA  GAACGCTTCA  GTGAGCTGGA
GCATCTGGAC  AGGCTCTCGG  GGAGGAGCTG  CTCGGAGGAG  AAGATTCCTG
AAGACGGCTC  CCTAAACACT  ACCAAATAGC  TCTTATGGGG  CAGGCTGGGC
ATGTCCAAAG  AGGCTGCCCC  TCTCACCAAA
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FIGURE 3

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MGRGLLRGLW   PLHIVLWTRI   ASTIPPHVQK   SVNNDMIVTD   NNGAVKPPQL
CKFCDVRFST   CDNQKSCMSN   CSITSICEKP   QEVCVAVWRK   NDENITLETV
CHDPKLPYHD   FILEDAASPK   CIMKEKKKPG   ETFFMCSCSS   DECNDNIIFS
EEYNTSNPDL   LLVIPQVTGI   SLLPPLGVAI   SVIIIFYCYR   VNRQQKLSST
WETGKTRKLM   EFSEHCAIIL   EDDRSDISST   CANNINHNTE   LLPIELDTLV
GKGRFAEVYK   AKLKQNTSEQ   FETVAVKIFP   YEEYASWKTE   KDIFSDINLK
HENILQFLTA   EERKTELGKQ   YWLITAPHAK   GNLQEYLTRH   VISWEDLRKL
GSSLARGIAH   LHSDHTPCGR   PKMPIVHRDL   KSSNILVKND   LTCCLCDFGL
SLRLDPTLSV   DDLANSGQVG   TARYMAPEVL   ESRMNLENAE   SFKQTDVYSM
ALVLWEMTSR   CNAVGEVKDY   EPPFGSKVRE   HPCVESMKDN   VLRDRGRPEI
PSFWLNHQGI   QMVCETLTEC   WDHDPEARLT   AQCVAERFSE   LEHLDRLSGR
SCSEEKIPED   GSLNTTK
```

I. CLASSIFICATION OF SUBJECT MATTER   (if several classification symbols apply, indicate all)[6]
According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12;   C12N5/10;   C12P21/08;   C07K13/00
A61K37/02

II. FIELDS SEARCHED
Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; A61K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[o]	Citation of Document, [11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP,A,0 369 861 (ONCOGEN LTD PARTNERSHIP, US) 23 May 1990 see the whole document	1-41
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 179, no. 1, 30 August 1991, DULUTH, MINNESOTA US pages 378 - 385 O'GRADY P;HUANG SS;HUANG JS; 'Expression of a new type high molecular weight receptor (type V receptor) of transforming growth factor beta in normal and transformed cells.' see the whole document	1,16,29

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20 JANUARY 1993	12. 02. 93

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	S.A. NAUCHE

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category°	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	CELL. vol. 68, no. 4, 21 February 1992, CAMBRIDGE, NA US pages 775 - 785 LIN HY;WANG XF;NG-EATON E;WEINBERG RA;LODISH HF; 'Expression cloning of the TGF-beta type II receptor, a functional transmembrane serine/threonine kinase.' see the whole document ---	1-3,6,7, 10,11, 14-16, 19,20, 24-26, 28-31 37-41
P,X	CELL. vol. 67, no. 4, 15 November 1991, CAMBRIDGE, NA US pages 785 - 795 LOPEZ-CASILLAS F;CHEIFETZ S;DOODY J;ANDRES JL;LANE WS;MASSAGUE J; 'Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system.' see the whole document ---	1-5,8,9, 12,13, 16-18, 21-23, 25-36,41
P,X	CELL. vol. 67, no. 4, 15 November 1991, CAMBRIDGE, NA US pages 797 - 805 WANG XF;LIN HY;NG-EATON E;DOWNWARD J;LODISH HF;WEINBERG RA; 'Expression cloning and characterization of the TGF-beta type III receptor.' see the whole document ---	1-5,8,9, 12,13, 16-18, 21-23, 25-36,41
A	JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS) vol. 265, no. 33, 25 November 1990, BALTIMORE, MD US pages 20533 - 20538 CHEIFETZ S;HERNANDEZ H;LAIHO M;TEN DIJKE P;IWATA KK;MASSAGUE J; 'Distinct transforming growth factor-beta (TGF-beta) receptor subsets as determinants of cellular responsiveness to three TGF-beta isoforms.' ---	
A	JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS) vol. 263, no. 32, 15 November 1988, BALTIMORE, MD US pages 16984 - 16991 CHEIFETZ S.; ANDRES J.L.; MASSAGUE J; 'The transforming growth factor-beta receptor type III is a membrane proteoglycan. Domain structure of the receptor.' ---	

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES vol. 593, 1990, NEW YORK, US; pages 59 - 72 MASSAGUE, J. ET AL; 'TGF-Beta recepotrs and TGF-beta binding proteoglycans : recent progress in identifying their functional properties.' -----	

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.   20/01/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0369861	23-05-90	CA-A- 2002011	14-05-90